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LebZIP2 induced by salt and drought stress and transient overexpression by *Agrobacterium*

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The full-length cDNA of LebZIP2 (*Lycopersicon esculentum* bZIP2) encodes a protein of 164 amino acids and contains a N-terminal basic-region leucine zipper domain. Analysis of the deduced tomato LebZIP2 amino acid sequence revealed that it shares 85% sequence identity with both tobacco bZIP and pepper CcbZIP. LebZIP2 mRNA is expressed at a high level exclusively in flowers. Presently, LebZIP2 was strongly increased also following NaCl and mannitol treatments. No significant LebZIP2 expression was evident following cold treatment. Transient LebZIP2 overexpression resulted in increased NbNOA1 and NbNR transcript levels in *Nicotiana benthamiana* leaves. Our results indicate that LebZIP2 might play roles as an abiotic stress-signaling pathway and as a transcriptional regulator of the NbNOA1 or NbNR genes. [BMB reports 2008; 41(10): 693-698]

INTRODUCTION

Basic leucine zipper (bZIP) proteins are one of the largest transcription factor families in plants (1). The bZIP domains form two parallel α-helices arranged as coils, which enable dimerization of two proteins (2). More than 80 bZIP proteins have been found in the *Arabidopsis* genome project (3). Interactions of each bZIP factor have been determined from the distribution of the bZIP region and the identities of the available partners in a given tissue (4).

The functional role of bZIP transcription factors has been linked to responses to light (5). The bZIP transcription factors are involved in plant defense, plant senescence, responses to various environmental stresses, and developmental processes (6). One of the bZIP proteins related to stress responses is comprised of TGA protein, which regulates the expression of some stress-responsive genes (7). Some bZIP factors have been associated with auxin, gibberellin, or abscisic acid (ABA) , and one group of bZIP factors mediates ABA-regulated gene expression (8). bZIP transcription factors are also involved in plant development and morphogenesis (9). For example, tobacco BZI-1 is associated with flower development (10).

Nitric oxide synthase (NOS)-like activity has been identified in soybean and pathogen interactions (11). Nitric oxide (NO)-associated 1 (NOA1) is detected as a factor related to NO production in *Arabidopsis thaliana* (At) (12). However, AtNOA1 has no sequence similarity with NOS of animals, and does not show any NOS-like activity itself. It has been suggested that NOA1 can be expected to be one of the genes involved in a nitrate reductase (NR)-independent NO-generation system (12).

In the present study, we employed reverse transcriptase-polymerase chain reaction (RT-PCR) to characterize a tomato bZIP transcription factor expressed in tomato leaves in response to elicitors related to environmental stresses. To better understand tomato bZIP gene functions, we also analyzed the tissue-specific expression of LebZIP2. The results suggest that the LebZIP2 gene might play a role in abiotic stress responses in tomatoes. Finally, we show that LebZIP2 can be a transcriptional regulator of NbNOA1 or NbNR genes by *Agrobacterium*-mediated transient overexpression.

RESULTS AND DISCUSSION

Sequence analysis of tomato LebZIP2

The sequence homologies among six bZIP transcription factors including LebZIP2 were analyzed (Fig. 1A). LebZIP2 was most similar in sequence to pepper CcbZIP (85% identity, GenBank Accession No. AAD21199) and tobacco NtbZIP (85% identity, GenBank Accession No. AAK92213). LebZIP2 was found to also share 66% sequence identity with soybean GmbZIP (GenBank Accession No. ABI34666) and 64% sequence identity with *Vitis* VvbZIP (GenBank Accession No. CAN73127). Heterodimerization of the BZI transcription factor, which in-

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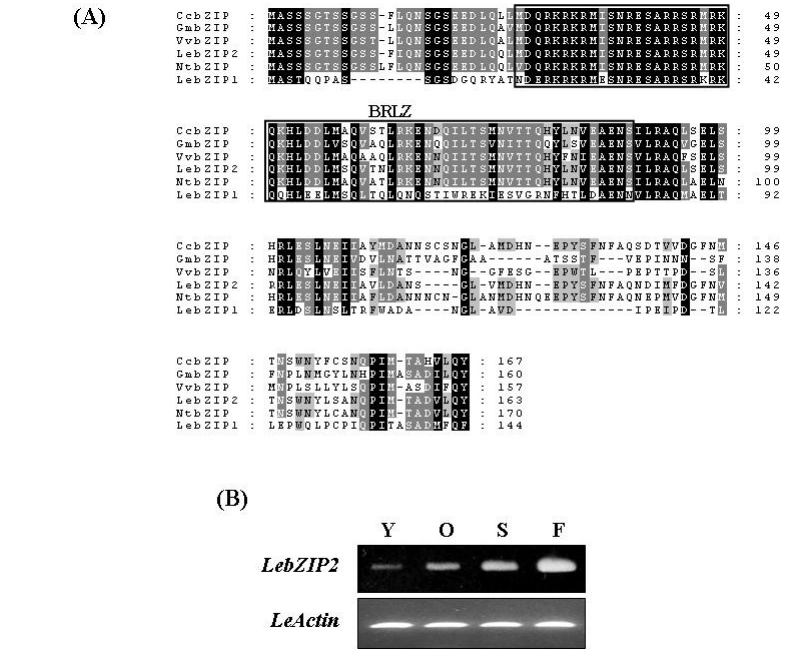

Fig. 1. Comparative analysis of derived amino acid sequences and organ-specific expression of tomato LebZIP2 gene. (A) Residues shaded in black are identical between the six proteins. The GenBank and NCBI accession numbers of peptide sequences are: *Capsicum chineses* AAD 21199 (CcbZIP), *Nicotiana tabacum* AA K92213 (NtbZIP), *Glycine max* ABI34666 (GmbZIP), *Vitis vinifera* CAN73127 and *Lycopersicon esculentum* AAD55394 (LebZIP1). The BRLZ is boxed-in. (B) *LebZIP2* expression in various pepper plant organs. LebZIP2 RNA accumulation in young leaf (Y), old leaf (O), stem (S), and flower (F).

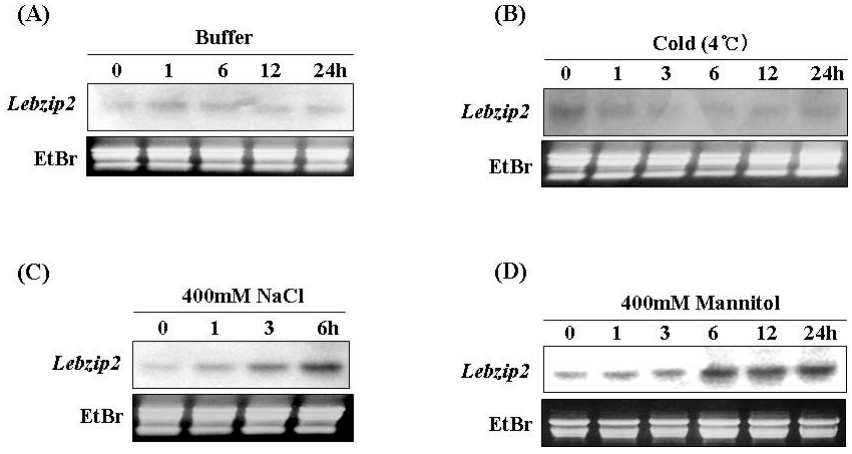

Fig. 2. Expression patterns of *LebZIP2* by environmental stresses. Tomato leaves were exposed to (A) buffer, (B) cold (4°C), (C) 400 mM NaCl and (D) 400 mM mannitol. Gel blot of tomato leaf total RNA hybridized with the [32P]-labeled LebZIP2 cDNA as a full length probe. rRNA bands in ethidium bromide-stained gels are shown as a loading control.

cludes a bZIP domain, may regulate several genes involved in plant development (10). Similar to bZIP transcription factors, expression of *LebZIP1*, which encodes a DNA-binding protein, is up-regulated by cold and dark treatment (13). Moreover, ABA prevents the rapid wound-induced increase in *LebZIP1* transcription, and the basal level of *LebZIP1* transcription is higher in ABA mutants (13); however, the functions of other genes with homology to *LebZIP1* have not been reported in detail. The LebZIP2 polypeptide is composed of 164 amino acids. The deduced amino acids of LebZIP2 cDNA are indicative of a bZIP transcription factor with a basic region leucine zipper (BRLZ) domain (26-90; Fig. 1). The LebZIP2 protein is highly homologous to other previously cloned plant bZIP DNA-binding proteins including those from tobacco (14), snapdragon (15), rice (16), maize (17), and *Arabidopsis* (18). Comparison of the deduced DNA-binding domains of LebZIP2 and LebZIP1 (GenBank Accession No. AF176641) revealed only a 45% identity at the amino acid level.

LebZIP2 transcription was highly expressed in flower tissues (Fig. 1B), consistent with observations that another homologous group of bZIPs are also transcriptionally activated after stress treatment or are specifically expressed in certain parts of the flower (10, 14, 15).

LebZIP2 induction by NaCl and mannitol

Fig. 2 shows the pattern of *LebZIP2* mRNA accumulation over

time in tomato plants exposed to a range of abiotic stresses including cold, high salinity, and mannitol. The expression patterns after cold stress did not differ in treated and untreated tomato leaves (Fig. 2B). After salt (NaCl) and drought stress (mannitol) treatments, the transcript levels of *LebZIP2* increased generally (Figs. 2C and 2D). Changes in gene expression in response to abiotic stresses such as drought, salinity, cold temperature, and high temperature vary based on the type and extent of stress (19). bZIP transcription factors play important roles in diverse biological processes including stress signaling and floral development (20). The bZIP transcription factor OsABI5 is involved in the response to stress and ABA signal transduction (21). OsABI5 is not induced by drought stress in rice (21). Based on these results, we propose that all genes involved in ROS signaling are unaffected by multiple stresses. The transcripts of *CAbZIP1* are induced by drought and salt stress, which indicates that *CAbZIP1* might also be involved in tolerance to environmental stresses (22). The tomato bZIP protein might play an important role in mediating transcriptional activation during wounding responses (13). In one study, strong *CAbZIP1* gene expression occurred at the early stage of infection in the incompatible interactions (22). Salicylic acid, ethylene and jasmonic acid are involved in disease resistance in plants (23, 24).

Expression of LebZIP2 in response to oxidative and osmotic stresses

To simulate oxidative stress, tomato plants were exposed to 50 μM methyl viologen. LebZIP2 gene expression was induced after 3 h and 6 h (Fig. 3A). The responses of LebZIP2 to hydrogen peroxide and ABA were the same as the results of MV treatment (Figs. 3B and 3C). The transgenic lines of overexpressed *CAbZIP1* are resistant to oxidative stress (25), and *CAbZIP1* overexpression can enhance the tolerance to stress by regulating the scavenging enzymes (26, 27). Survival of plants involved in efficient anti-oxidizing systems under serious environmental conditions has been studied (28). Two bZIP transcription factors have been cloned and demonstrated capable of interaction with the ABA responsive element (ABRE) (29). The rab28 promoter is important to the induction of expression for ABA and stress (30, 31). The other transcription factor, ZmBZ-1, is involved in rice OsBZ8 (32). The ABRE binding factor and the ABRE binding proteins can bind to different ABRE-containing promoters *in vitro* and in yeast (33, 34). AtbZIP39/ABI5, AtbZIP36/ABF2/AREB1, AtbZIP38/ABF4/AREB2, AtbZIP66/AREB3, AtbZIP40/GBF4, AtbZIP35/ABF1, and AtbZIP37/ABF3 have been studied for their roles in ABA response or signaling in response to stresses such as cold, drought, and high salinity (8, 33-35). Induction of ABA after various stimuli can evoke the expression of defense proteins such as Pin2 as well as specific transcription factors such as bZIP (8).

Transient expression by *Agrobacterium*

We investigated the role of LebZIP2 in the response to abiotic and biotic stress. To demonstrate whether this transcription factor could contribute to the expression of genes related to defense and nitric accumulation, *Agrobacterium tumefaciens* strain LBA4404 harboring pMBP1 binary vectors with a coding region of LebZIP2 under the control of the CaMV 35S promoter, or vector only, was infiltrated into leaves of *N. benthamiana*. After 1 day, the total RNA from the *N. benthamiana* leaves was extracted for RT-PCR analysis. When LebZIP2 was overexpressed in tobacco leaves, NbHSR203J, NbPR1a, NbPAL and NbSIP gene transcripts did not vary as compared with control plants harboring only the vector (Fig. 4). However, LebZIP2 transiently increased the expression of NOA1 and NR genes in the overexpressed leaves (Fig. 4). The nitrate-nitrite pathway catalyzed by NR and NOS inhibitors affect NO generation systems in plants (36). NO triggers defense-related gene expression and hypersensitive response (37, 38). Silencing of NbNOA1 af-

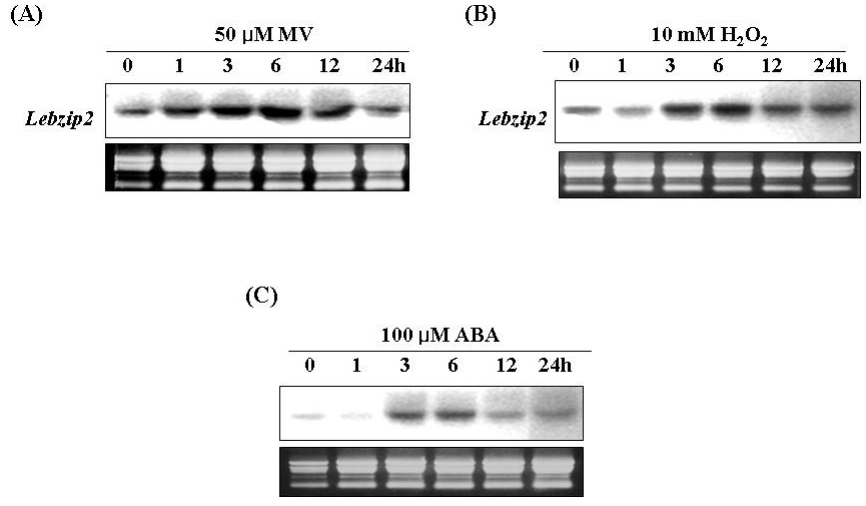

Fig. 3. Expression patterns of *LebZIP2* in tomato leaf tissues treated with abiotic elicitors. (A) 50 μM methyl viologen (MV); (B) 10 mM hydrogen peroxide (H_2O_2); (C) 100 μM ABA. Total RNA from the leaf tissues at various time intervals after treatment was loaded in each lane. A duplicate gel was stained with ethidium bromide as a control for RNA loading.

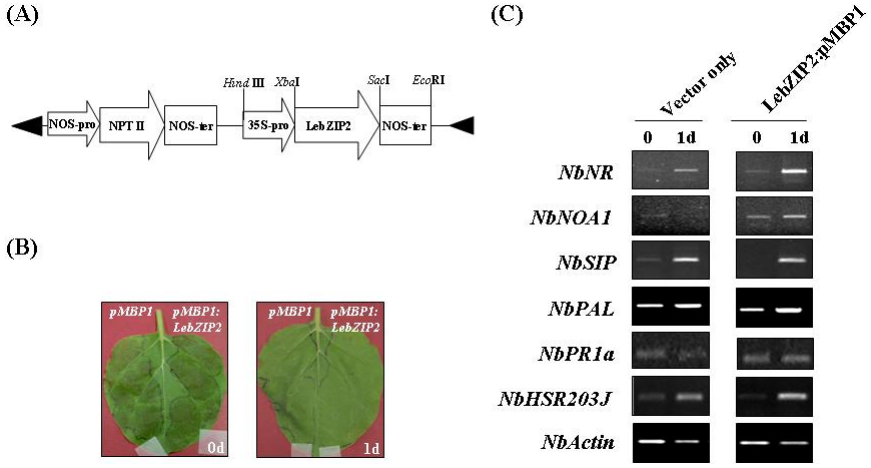

Fig. 4. Vector construction of LebZIP2: pMBP1 to transient overexpression in leaves of *N. benthamiana*. (A) Construction of *LebZIP2* in the pMBP1 vector. (B) Tobacco leaves that were infiltrated with vector only, or which overexpressed LebZIP2 by *Agrobacterium*-mediated transient transformation (0 day and 1 day, respectively). (C) Expression of various genes activated by transient LebZIP2 overexpression in tobacco leaves. The expression of four pathogen-related genes (*NbHSR203J, NbPR1a, NbPAL,* and *NbSIP*) and two nitrate accumulation-related genes (*NbNR* and *NbNOA1*) were compared by RT-PCR. NbActin was used as a positive control. Primers used were previously described (39). The experiment was repeated at least three times and a representative result is shown.

fects PR1a gene expression, whereas expression is not affected in HSR203J (39). By contrast, NO production is responsible for the expression of hsr genes (40). *NbNOA1* is necessary for defense responses and is involved in NO production (39). *HY5* and *HYH* as well as the bZIP transcription factors are involved in photomorphogenesis signaling, with the genes being essential for induction of NR, a key enzyme in nitrogen assimilation (41). *HY5* and *HYH* are also essential for strong expression of NR in seedlings as well as rosette-stage plants (42). A more detailed analysis of LebZIP2 is necessary to any extensive understanding of the overexpressed transgenic plant in the transcriptional regulatory network.

We investigated several both abiotic and biotic stress signaling-related genes in *N. benthamiana*. The genes have been previously shown to have antioxidant effects and to play roles in defense and nitrate accumulation. We screened for genes that regulate *LebZIP2* by means of the transient expression of the gene. *LebZIP2* expression was induced by ROS production, but did not differ based on the expression of the ROS scavengers APX and SOD. Two genes known to be involved in nitrate accumulation (*NR* and *NOA*) were up-regulated in the *Agrobacterium*-infiltrated *N. benthamiana* leaves. Interestingly, these genes are involved in plant defense (36).

In this study, we provide new evidence concerning the function of LebZIP2 during plant stress. The role of LebZIP2 in salt and drought stress signaling was confirmed and its involvement in stress tolerance is indicated.

MATERIALS AND METHODS

Isolation of *LebZIP2* from tomato

We searched for and analyzed full-length nucleotide and deduced amino acid sequences to obtain the tomato bZIP2 gene clone (http://www.sgn.cornell.edu/). Full-length primers were designed to amplify the *LebZIP2* of tomato. The primer sequences were 5′-ATGGCTTCGTCAAGTGGT-3′ (forward) and 5′-TCAGTACTGCAAGACATC-3′ (reverse). The band detected by PCR amplification was cloned into the pGEM T Easy vector (Promega, Madison, WI). We confirmed that the obtained sequences matched those at http://www.sgn.cornell.edu/.

Chemical treatments of tomato

'MicroTom' tomato seeds were inoculated on MS medium (MS salts, 3% sucrose, 0.8% agar, pH 5.8). After 2 weeks, germinated plants were transferred to pots and kept in a growth chamber at 28°C for 4 weeks. Various abiotic elicitors were applied to the leaves of the tomato plants. The plants were placed in a solution containing 400 mM NaCl, 400 mM mannitol and 50 μM methyl viologen. The leaves of some whole plants were sprayed with 100 μM ABA and 10 mM hydrogen peroxide. For cold treatment, they were placed in distilled water and kept in a 4°C cold room under dim lighting for 8 h or 24 h. The treated leaves were frozen in liquid nitrogen and stored at −70°C until used for RNA extraction.

RNA preparation

Total RNA was extracted from various tomato tissues (young leaves, old leaves, stems, flowers) as previously described (43). Five ml of Trizol reagent (Invitrogen, Carlsbad, CA) were then added to 1 g of plant materials that had been frozen in liquid nitrogen and ground to a powder. The mixture was incubated for 5 min at room temperature and chloroform was added. The samples were centrifuged and the supernatant was transferred to a new tube. This process was repeated. Precipitation was performed with isopropanol at −20°C for 1 h. After centrifugation, the pellet was washed in 70% ethanol and resuspended in diethylpyrocarbonate water.

RNA gel blot hybridization and RT-PCR

For Northern-blot analyses, total RNA was separated on formaldehyde containing agarose gels and blotted onto nylon membranes following standard procedures (44). Equal loading of RNA

was verified by visualizing of rRNA following staining with ethidium bromide. Blots were hybridized with [a-32P]dCTP-labeled probes (LebZIP2).The amplified DNA fragments were cloned into a pGEM-T easy vector (Promega) and the determination of the correct gene was confirmed. First-strand cDNA was synthesized from 1 μg samples of DNase-treated total RNA (M-MLV RT, Invitrogen). The primers used for RT-PCR were as follows: (5′-ATGGCTTCGTCAAGTGGT-3′ and 5′-TCAGTACTGCAAGA CATC-3′) for *LebZIP2*. Other gene-specific primers for each sequence were: NbActin forward primer, 5'-CAGCTCATCCGTGG AGAAGA-3', NbActin reverse primer, 5'-AGGATACGGGGAG CTAATGC-3', NbHSR203J forward primer, 5'-TGTGTCAGCCA TGCTGATTG-3', NbHSR203J reverse primer, 5'-CCGATAGGA CCGCACGAAAC-3', NbPR1a forward primer, 5'-ATGGGATTT GTTCTCTTTTC-3', NbPR1a reverse primer, 5'-TTAGTATGGAC TTTCGCCTC-3', NbPAL forward primer, 5'-TCGAGTTGCAGC CTAAGG-3', NbPAL reverse primer, 5'-TCTTCCAAATGCCTCA AGTC-3', NbSIP forward primer, 5'-TGAGTCAGGCAGGAAGC AAG-3', NbSIP reverse primer, 5'-TTTGTACAAGAAAGGTGG-3', NbNOA1 forward primer, 5'-GTTACTGCAGAAGAGCTTCG-3', NbNOA1 reverse primer, 5'-TATCATACACTTTCCCACCG-3', NbNR forward primer, 5'-CCCTCTGAGGATCAAGTCTTG-3', and the NbNR reverse primer, 5'-CATAGCCCATCTTCTCCAA G-3'. PCR reactions constituted an initial denaturation for 5 min at 94°C, 25 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, and a final 7 min extension at 72°C. Aliquots (12 μl) of the reaction products were separated on 1% agarose gels and visualized by staining with ethidium bromide. All experiments were performed in triplicate.

Transient expression of LebZIP2 in *N. benthamiana*

For agroinfiltration experiments, LebZIP2 were inserted into *Xba*I-*Sac*I sites of the vector pMBP1. The binary plasmid was transformed into *A. tumefaciens* strain LBA4404 (45). *Agrobacterium* cells were harvested by centrifugation and suspended in 10 mM MES-NaOH (pH 5.6), 10 mM $MgCl_2$, and 100 μM acetosyringone to an optical density (600 nm) of 0.7, incubated at room temperature for 2 h, then used to infiltrate leaves of *N. benthamiana* using a needle-free syringe.

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REFERENCES

1. Landschulz, W. H., Johnson, P. F. and McKnight, S. L. (1988) The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* **240**, 1759-1764.
2. Baxevanis, A. D. and Vinson, C. R. (1993) Interactions of coiled coils in transcription factors: where is the specificity? *Curr. Opin. Genet. Dev.* **3**, 278-285.
3. Riechmann, J. L., Heard, J. and Martin, G. (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science* **290**, 2105-2110.
4. Hurst, H. C. (1995) Transcription factors. 1. bZIP proteins. *Protein Profile* **2**, 105-168.
5. Oyama, T., Shimura, Y. and Okada, K. (1997) The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes Dev.* **11**, 2983-2995.
6. Jakoby, M., Weisshaar, B., Droge-Laser, W., Tiedemann, J., Kroij, T. and Parcy, F. (2002) The family of bZIP transcription factors in *Arabidopsis thaliana*. *Trends Plant Sci.* **7**, 106-111.
7. Despres, C., Chubak, C., Rochon, A., Clark, R., Bethune, T., Desveaux, D. and Fobert., P. (2003) The Arabidopsis NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. *Plant Cell* **15**, 2181-2191.
8. Finkelstein, R. and Lynch, T. (2000) The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. *Plant Cell* **12**, 599-609.
9. Heinekamp, T., Kuhlmann, M., Lenk, A., Strathmann, A. and Droge-Laser, W. (2002) The tobacco bZIP transcription factor BZI-1 binds to G-box elements in the promoters of phenylpropanoid pathway genes *in vitro*, but it is not involved in their regulation *in vivo*. *Mol. Genet. Genomics* **267**, 16-26.
10. Strathmann, M., Kuhlmann, M., Heinekamp, T. and Dröge-Laser, W. (2001) BZI-1 specifically heterodimerises with the tobacco bZIP transcription factors BZI-2, BZI-3/TBZF and BZI-4, and is functionally involved in flower development. *Plant J.* **28**, 397-408.
11. Modolo, L. V., Cunha, F. Q., Braga, M. R. and Salgado, I. (2002) Nitric oxide synthase-mediated phytoalexin accumulation in soybean cotyledons in response to the *Diaporthe phaseolorum* f. sp. meridionalis elicitor. *Plant Physiol.* **130**, 1288-1297.
12. Crawford, N. M., Galli, M., Tischner, R., Heimer, Y. M., Okamoto, M. and Mack, A. (2006) Plant nitric oxide synthase: back to square one. *Trends in Plant Sci.* **11**, 526-527.
13. Stankovic, B., Vian, A., Henry-Vian, C. and Davies, E. (2000) Molecular cloning and characterization of a tomato cDNA encoding a systemically wound-inducible bZIP DNA-binding protein. *Planta* **212**, 60-66.
14. Kusano, T., Sugawara, K., Harada, M. and Berberich, T. (1998) Molecular cloning and partial characterization of a tobacco cDNA encoding a small bZIP protein. *Biochim. Biophys. Acta.* **1395**, 171-175.
15. Martinez-Garcia, J. F., Moyano, E., Alcocer, M. J. and Martin, C. (1998) Two bZIP proteins from Antirrhinum flowers preferentially bind a hybrid C-box/G-box motif and help to define a new subfamily of bZIP transcription factors. *Plant J.* **13**, 489-505.
16. Zou, M., Guan, Y., Ren, H., Zhang, F. and Chen, F. (2008) A bZIP transcription factor, OsABI5, is involved in rice fertility and stress tolerance. *Plant Mol. Biol.* **66**, 675-683.
17. Singh K., Dennis, E. S., Ellis, J. G., Llewellyn, D. J. Tokuhisa., J. G., Wahleithner, J. A. and Peacock, W. J. (1990) OCSBF-1, a maize ocs enhancer binding factor: isolation and expression during development. *Plant Cell* **2**, 891-903.

18. Rook, F., Weisbeek, P. and Smeekens, S. (1998) The light-regulated Arabidopsis bZIP transcription factor gene ATB2 encodes a protein with an unusually long leucine zipper domain. *Plant. Mol. Biol.* **37**, 171-178.
19. Aguan, K., Sugawara, K., Suzuki, N. and Kusano, T. (1993) Low temperature-dependent expression of a rice gene encoding a protein with a leucine-zipper motif. *Mol. Gen. Genet.* **240**, 1-8.
20. Sreenivasulu, N., Sopory, S. K. and Kavi Kishor, P. B. (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. *Gene* **388**, 1-13.
21. Finkelstein, R. R., Gampala, S. S. and Rock, C. D. (2002) Abscisic acid signaling in seeds and seedlings. *Plant Cell* **14**, S15-S45.
22. Lee, S. C., Choi, H. W., Hwang, I. S., Choi, D. S. and Hwang, B. K. (2006) Functional roles of the pepper pathogen-induced bZIP transcription factor, CAbZIP1, in enhanced resistance to pathogen infection and environmental stresses. *Planta* **224**, 1209-1225.
23. Ukness, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E. and Ryals, J. (1992) Acquired resistance in Arabidopsis. *Plant Cell* **4**, 645-656.
24. Rickauer, M., Brodschelm, W., Bottin, A., Veronesi, C., Grimal, H. and Esquerre-Tugaye, M. T. (1997) The jasmonate pathway is involved differentially in the regulation of diVerent defense responses in tobacco cells. *Planta* **202**, 155-162.
25. Asada, K. (1999) The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 601-639.
26. Karpinski, S., Wingsle, G., Karpinska, B., Hallogren, J. E. (2001) Redox sensing of photooxidative stress and acclamatory mechanisms in plants; In *Regulation of Photosynthesis*, Aro, E. M. and Andersson, B. (eds.), pp 469-486, Kluwer, Dordrecht.
27. Murgia, I., Tarantino, D., Vannini, C., Bracale, M., Carravieri, S. and Soave, C. (2004) *Arabidopsis thaliana* plants overexpressing thylakoidal ascorbate peroxidase show increased resistance to paraquat-induced photooxidative stress and to nitric oxideinduced cell death. *Plant J.* **38**, 940-953.
28. Kranner, I., Beckett, R. P., Wornik, S., Zorn, M. and Pfeifhofer, H. W. (2002) Revival of a resurrection plant correlates with its antioxidant status. *Plant J.* **31**, 13-24.
29. Claudia, N., Busk, P. K., Domínguez-Puigjaner, E., Lumbreras, V., Testillano, P. S., Risueň, M. C. and Pagès, M. (2005) Isolation and functional characterisation of two new bZIP maize regulators of the ABA responsive gene rab28. *Plant Mol. Biol.* **58**, 899-914.
30. Pla, M., Vilardell, J., Guiltinan, M. J., Marcotte, W. R., Niogret, M. F., Quatrano, R. S. and Pagès, M. (1993) The cis-regulatory element CCACGTGG is involved in ABA and waterstress responses of the maize gene rab28. *Plant Mol. Biol.* **21**, 259-266.
31. Busk, P. K., Pujal, J., Jessop, A., Lumbreras, V. and Pagès, M. (1999) Constitutive protein-DNA interactions on the abscisic acid-responsive element before and after developmental activation of the rab28 gene. *Plant Mol. Biol.* **41**, 529-536.
32. Nakagawa, H., Ohmiya, K. and Hattori, T. (1996) A rice bZIP protein, designated OSBZ8, is rapidly induced by abscisic acid. A bZIP factor, TRAB1, interacts with VP1 and mediates abscisic acid-induced transcription. *Plant J.* **9**, 217-227.
33. Choi, H., Hong, J., Ha, J., Kang, J. and Kim, S. Y. (2000) ABFs, a family of ABA responsive element binding factors. *J. Biol. Chem.* **275**, 1723-1730.
34. Uno, Y., Furihata, T., Abe, H., Yoshida, R., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000) *Arabidopsis* basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 11632-11637.
35. Lopez-Molina, L. Mongrand, S. and Chua, N. H. (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4782-4787.
36. Yamamoto-Katou, A., Katou, S., Yoshioka, H., Doke, N. and Kawakita, K. (2006) Nitrate reductase is responsible for elicitin-induced nitric oxide production in Nicotiana benthamiana. *Plant Cell Physiol.* **47**, 726-735.
37. Grun, S., Lindermayr, C., Sell, S. and Durner, J. (2006) Nitric oxide and gene regulation in plants. *J. Exp. Botany* **57**, 507-516.
38. van Loon, L. C., Rep, M. and Pieterse, C. M. J. (2006) Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* **44**, 135-162.
39. Kato, H., Asai, S., Yamamoto-Katou, A., Yoshioka, H., Doke, N. and Kawakita, K. (2008) Involvement of NbNOA1 in NO production and defense responses in INF1-treated *Nicotiana benthamiana*. *J. Gen. Plant Pathol.* **74**, 15-23.
40. Yamamoto, A., Katou, S., Yoshioka, H., Doke, N. and Kawakita, K. (2004) Involvement of nitric oxide generation in hypersensitive cell death induced by elicitin in tobacco cell suspension culture. *J. Gen. Plant Pathol.* **70**, 85-92.
41. Chattopadhyay, S., Ang, L. H., Puente, P., Deng, X. W. and Wei, N. (1998). *Arabidopsis* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. *Plant Cell* **10**, 673-683.
42. Jonassen, E. M., Lea, U. S. and Lillo, C. (2008) *HY5* and *HYH* are positive regulators of nitrate reductase in seedlings and rosette stage plants. *Planta* **227**, 559-564.
43. Yi, S. Y., Kim, J. H., Joung, Y. H., Lee, S., Kim, W. T., Yu, S. H. and Choi, D. (2004) The pepper transcription factor CaPF1 confers pathogen and freezing tolerance in Arabidopsis. *Plant Physiol.* **136**, 2862-2874.
44. Sambrook, J, Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Plainview, NY
45. Hellens, R. P., Edwards, E. A., Leyland, N. R., Bean, S. and Mullineaux, P. M. (2000) pGreen: a versatile and flexible binary Ti vector for *Agrobacterium*-mediated plant transformation. *Plant Mol. Biol.* **42**, 819-832.